Subchronic Lead Feeding Study in Male Rats and Micropigs

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ABSTRACT: This study compared the lead uptake from contaminated test soil of known lead concentration with a soluble lead acetate standard, which was considered to be 100% bioavailable. This study also compared the lead bioavailability from this lead-contaminated soil between rats and micropigs. Harlan Sprague–Dawley rats and Yucatan micropigs were fed lead-contaminated soil as a 5% (w/w) mixture with their diet. The lead-contaminated soil was either a specific test soil of known lead concentration (1000 μg/g) or basal low concentration lead soil ($\sim$135 μg/g), which was spiked with lead acetate to match the lead content of the test soil. The effective diet lead concentration was 50 μg Pb/g diet. Results demonstrated that rats reached steady-state concentrations of blood lead by week 2, whereas micropigs did not reach steady state until week 4. In addition, final blood lead concentrations in micropigs were four-fold higher than those in rats. In the micropigs, the final blood lead levels in the test soil study group were significantly lower than those in the lead acetate study group, although there was no significant difference between study groups in the rats. Tissue lead concentrations were significantly higher in micropigs than those in the rats, although the diet lead concentrations in both sets of animals were the same. In summary, because of the greater sensitivity in demonstrating higher tissue lead incorporation in micropigs as compared to rats, the micropig is a better animal model for demonstrating the differences in relative lead bioavailability when testing different sources of lead-contaminated soils. Environ Toxicol 24: 453–461, 2009.

Keywords: animal models; feeding study; lead absorption; bioavailability

INTRODUCTION

The ingestion of lead-contaminated soil and dust incidental to hand-to-mouth activity presents one of the principal pathways for exposure to nondietary lead, especially in areas with significant soil contamination (Mielke and Reagan, 1998). Lead intoxication is a matter of health concern, primarily for young children. Therefore, the problem is to determine an appropriate animal model, which simulates the human condition, both physiologically and developmentally, in which we could test various lead remediation procedures.

As described in a previous report by Weis and LaVelle (1991), rodents and lagomorphs do not quite fulfill the basic requirements of gastrointestinal physiology and biochemistry of young children. However, because of the ease and low cost of using these animals for research purposes, many of the regulatory guidelines for humans have been established using these animal models. Scientific evidence,

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however, suggests that for various physiological and biochemical processes, such as nutrition, gut immunology, and cardiovascular responses, pigs may be more physiologically relevant to the human condition (Dodds, 1982; Miller and Ulrey, 1987) than do rats.

This particular study was designed to test which animal model (rat *vs*. micropig) allows us to better measure the lead uptake from the intestinal lumen into the blood stream. Furthermore, we compared the lead uptake from a test soil of known lead concentration (1000 μg/g Pb) to a lead acetate-spiked soil of similar dosage. Lead acetate was used as the standard reference, which was considered to be 100% bioavailable at the time of study. This allowed us to compare the lead relative bioavailability of our test soil between rats and micropigs.

METHODS

Test soils were obtained from Hazen Research, Golden, Colorado (Project No. 7910-08). These samples were taken from Weyerhaeuser sites in Tacoma, Washington. Pb-contaminated soils are presumably due to smelter emissions. Test soil names (and reported lead concentrations) used in the study were labeled "S" (135 μg Pb/g) and "P" (1000 μg Pb/g). Analytical characterizations on soil samples for this lead contamination bioavailability study were performed by Hazen Research. Soil samples were dried overnight to facilitate particle size separation. All soil samples were screened to -60 mesh (250 μm particle size). Soil lead concentration was determined by ICP-AA. The compound lead acetate (Sigma Cat. No. L-3396) was obtained from Sigma Chemical Company and was used as an addition to the basal "control" soil "S" (135 μg Pb/g) to adjust the total lead concentrations to the target concentration of 1000 μg Pb/g.

All procedures utilized in this study were previously approved by the Louisiana State University Medical Center, New Orleans, Institutional Animal Care and Use Committee. Eighteen (18) male weanling Sprague–Dawley rats, $\sim$21 days of age, with body weights ranging from 30 to 60 g were acquired from Harlan Sprague–Dawley, Indianapolis, IN. The rodents were housed in filtered shipping crates and delivered by Harlan climate-controlled trucks. Upon arrival, the animals were weighed, implanted subdermally (using aseptic techniques) with an animal identification microchip (American Veterinary Identification Devices, AVID, Norco, CA) at the nape of the neck, and placed in individual filter-top cages.

Eleven (11) male weanling Yucatan Micro-Pigs, $\sim$30 days of age, with body weights ranging from 3 to 6 kg were acquired from Charles River Laboratories, Wilmington, MD. The micropigs were housed in filtered shipping crates and delivered by climate-controlled trucks. Upon arrival, the animals were weighed, implanted subdermally (using aseptic techniques) with an animal identification microchip (American Veterinary Identification Devices, AVID, Norco, CA) at the nape of the neck, and placed in individual HEPA-filtered large animal isolation units (PlasLabs, Lansing, MI).

Each rat was housed singly in a filter-top polycarbonate plastic cage to prevent test animal exposure to environmental lead and cross exposure to other formulations of lead diet used in the study. Cage cleaning, weighing, and feeding studies were conducted in a laminar air-flow hood (Germfree Labs, Model BF-4) to protect laboratory personnel from lead exposure. Raised wire-mesh cage floors were used to prevent coprophagy. Cages were wiped clean daily using a 2% (v/v) Chlorox solution during the weighing and feeding procedure and sanitized once a week.

Each micropig was housed individually in HEPA-filtered, autoclavable, isolation units to prevent test animal exposure to environmental lead and cross exposure to other formulations of lead diet used in the study. Raised wire mesh floors were used to prevent coprophagy. Isolation units were scrubbed clean daily using a 2% (v/v) Chlorox solution and washed with hot water during the weighing and feeding procedure, and sanitized once a week. Isolation unit cleaning, weighing, and feeding procedures were conducted by laboratory personnel wearing whole-body, limited-use, disposable jump-suits, scrub boots, dust mask, head/hair bonnet, and rubber gloves to protect laboratory personnel from incidental lead exposure.

Animal rooms were targeted to a temperature of 23 $\pm$ 2°C and relative humidity of 50 $\pm$ 25%. Room temperatures and humidity were monitored using a 7-day temperature/humidity chart recorder (The Dickson Company, Addison, IL). The animal rooms were artificially illuminated (fluorescent lighting) on a 12-h light/dark cycle.

Animals were quarantined in their appropriate cages and animal rooms for a minimum of 6 days before the start of the study. During this period, rats were fed *ad libitum* purified Rat Basal (95%) Diet mix (Production Code TD 92242, powder) and micropigs were fed *ad libitum* purified Swine Basal (95%) Diet mix (Production Code TD 93034, powder) obtained from Harlan Teklad, Madison, WI. Animals were weighed daily and observed with respect to body weight gain and any gross signs of disease or injury. The animals were released from quarantine by the LSU institutional laboratory animal veterinarian on the basis of body weights and clinical evaluations at the end of the pretest period.

During the test period, rats and micropigs in each group (as identified in the Study Design) were fed lead-soil diet mixes prepared with either purified Rat Basal (95%) Diet mix (Production Code TD 92242, powder) or purified Swine Basal (95%) Diet mix (Production Code TD 93034, powder) obtained from Harlan Teklad, Madison, WI. These diet mixes were custom formulated by Harlan Teklad so that each diet contained complete 100% normal nutrition

when the diet was diluted to 95% with 5% (w/w) bulk test agents, i.e., test soils containing lead.

Lead acetate was dissolved into a vehicle of sterile, deionized H_2O and evenly atomized in one-third portions over the surface of the premixed soil "S"/diet mix and then blended for an additional 15 min. The test soil "P" (1000 μg Pb/g) diet mixtures were similarly atomized with sterile, deionized H_2O as a control vehicle. Therefore, the effective diet lead concentration was 50 μg Pb/g diet. After the diets were prepared, samples were collected from each concentration of diet prepared with lead-soil to verify lead concentration and diet homogeneity. Homogeneity samples were collected from the top, middle, and bottom of the diet mixer and frozen until analysis. Diet preparations were properly labeled (color-coded) and refrigerated until use.

The experimental design held the lead concentration constant at 50 μg Pb/g diet and varied the animal test species: rat *versus* micropigs for a 30-day test period. The soil amount was kept constant (5% w/w), while the lead compositions (lead acetate or test soil) were varied, resulting in four total study groups with five animals each.

All animals were first scanned for identification using the AVID microchip frequency scanner and the identity crosschecked with the cage identity for the appropriate animal number and dietary study group. All animals, and respective feeder jars (both initial weight and final filled weight), were weighed and recorded daily using the Multi-Weigh© software program. Food jars were refilled with the appropriate soil/diet mix when necessary to assure sufficient food consumption levels *ad libidum*. Rat body weight and feeder jar weighings were done in the laminar flow hood to protect the laboratory personnel from lead exposure. However, the micropigs and corresponding feeder jars were weighed using a large animal balance, A&D Model EP-20KA (A&D Engineering, Militas, CA), outside the laminar flow hood. For safety purposes, laboratory personnel were required to wear whole-body, limited-use, disposable jump-suits, scrub boots, dust mask, head/hair bonnet, and rubber gloves to protect the laboratory personnel from lead exposure.

For rats, total daily fecal samples were collected into 20-mL polypropylene collection vials and weighed. The sample vials were placed in a Fisher brand convection oven set at 60°C and allowed to dry overnight. When the final fecal weight data were analyzed, the appropriate wet weights were matched up with the corresponding dry weights, which were recorded on the following day. For rats, the entire fecal output for a corresponding 7-day period was pooled into a 50-mL conical centrifuge tube and cleaned from any contaminating pieces of paper bedding and spilled diet. The final weights were measured and recorded. From these pooled samples, total weekly lead excretion values were determined.

For micropigs, the total daily fecal mass was collected and weighed. However, only representative aliquots were collected into sample collection vials, weighed, and dried overnight at 60°C. When the fecal weight data were analyzed, the appropriate wet weights were matched up with the corresponding dry weights, which were recorded the following day. The dried fecal samples for a corresponding 7-day period were pooled and the final weights were measured and recorded. No cleaning of pooled micropig fecal samples, as was done with the rat fecal samples, was necessary before being analyzed for lead concentration. The micropig fecal wet/dry weight ratios were used to calculate the total fecal lead output.

As required by the study protocol, the five rats scheduled to be bled from the 1000 μg Pb/g lead acetate-soil diet study group were individually selected at random. All five pigs in the 1000 μg Pb/g lead acetate soil diet study group were scheduled for periodic bleed to determine blood lead concentration. Rats were bled via the optic orbital sinus using either heparinized or 1% EDTA solution-rinsed 50-μL glass capillary tubes. Micropigs were bled via the femoral artery using a 1-1/2" 20G needle affixed to a 10-mL syringe, which had been rinsed with a 1% EDTA solution. After bleeding, animals were observed for any ill side effects of the procedure before being returned to their appropriate cages.

After 30 days of continuous feeding, both rats and micropigs were sacrificed and bone (femur) and soft tissue samples (liver and kidney) were obtained. Cardiac blood was obtained during exsanguination at the time of final sacrifice and these blood samples were included with the indicated tissue samples to be sent for analysis of lead content. Samples of soil, diets, blood, and tissues were analyzed for total lead content. Final calculated results are presented as micrograms of lead per gram of food, tissue, bone, or soil (μg Pb/g dry weight). Final blood lead concentrations were calculated as micrograms of lead per deciliter (μg Pb/dL) of blood.

State-of-the-art instrumentation was used for the analysis of specimens. The Atomic Absorption Spectrophotometer is a GBC Instruments Model 908, which has flame (FLAA) and graphite electrothermal furnace (GFAA) capabilities, a computerized data processor and a PAL 3000 graphite furnace autosampler. An Applied Research Laboratories Model 3410 Inductively-Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES) with mini-torch and maximum dissolved solids nebulizer is used to analyze digested samples containing higher levels of lead and those samples that are digested with hydrochloric acid.

Blood samples collected in capillary tubes or vacutainer tubes containing sodium EDTA were stored under refrigeration. These were prepared for analysis by dilution 1:5 with a matrix modifier solution containing 10 g/L diammonium hydrogen phosphate and 1 mL/L Triton X-100 following the method of Sinclair (1988). Blood samples were analyzed by GFAA using matrix-matched standards and controls (University of Wisconsin, Department of Hygiene,

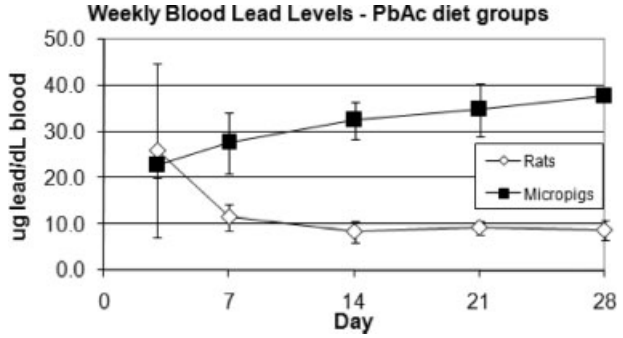

Fig. 1. Weekly blood lead levels—PbAc diet groups. Values represent the mean ± SD for each group of five animals. An * indicates that the values are significantly different between groups at $P < 0.05$ using Dunnett's test for statistical significance.

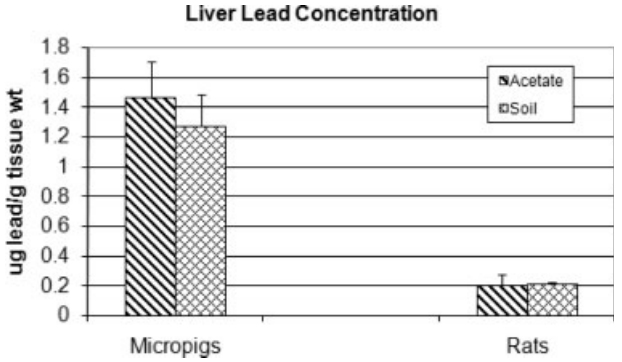

Fig. 3. Liver lead concentrations. Values represent the mean ± SD for each group of five animals. An * indicates that the values are significantly different between groups at $P < 0.05$ using Dunnett's test for statistical significance.

Madison, WI, and BioRad ECS Division, 3726 Miraloma Ave., Anaheim, CA). Using a furnace temperature program, 20 μL of sample (or standard) prediluted with matrix modifier solution was injected directly onto the coated graphite furnace tube. The resulting data were generated as integrated peak area absorbencies.

Bone samples (femur) were processed using a modification of the method by Witters et al. (1981). The bone was placed in a beaker that contained a known volume of 2 N NaOH and allowed to soak overnight to digest and residual soft tissue. A portion of the central shaft of bone was dried at 110°C to a constant weight and then ashed in a muffle furnace at 450°C for 48 h. The bone ash was ground in a mortar and a 25 mg portion was dissolved in nitric acid to which a lanthanum (853 μg La/mL) matrix modifier was added.

Liver and kidney tissue samples were subjected to a pressure-wet digestion procedure. Tissues were placed in two-ounce Nalgene bottles with 1 mL perchloric acid and 2 mL of concentrated nitric acid, and tightly sealed for ~8 h. The samples were then incubated in a hot water bath for 3 h, diluted with 3 mL distilled water, and reheated under a fume hood to evaporate the excess acid.

Samples of lead target soil, diet, and feces underwent a wet digestion procedure, such as EPA Method 3050 (Manual SW846), prior to analysis. In this procedure, specimens were digested by refluxing on a hot plate during a series of treatments with nitric acid, hydrogen peroxide, and hydrochloric acid solutions. The samples were then filtered and reconstituted to a volume of 50 mL with deionized water for analysis.

Final calculated results were presented as micrograms of lead per deciliter of blood (μg Pb/dL), or micrograms of lead per gram of food, tissue, bone, or soil (μg Pb/g dry wt).

Data for body weight, food consumption, and fecal output were collected daily for the entire 30-day period. These daily data were then grouped into weekly summaries (weeks 1–5). However, week 5 consisted only of 2 days: day 29 and day 30. Therefore, results of weekly blood level shown in Figure 1 have been simplified to show only weeks 1–4. Total values for fecal output and food consumption have been calculated for the entire 30-day test period. Body

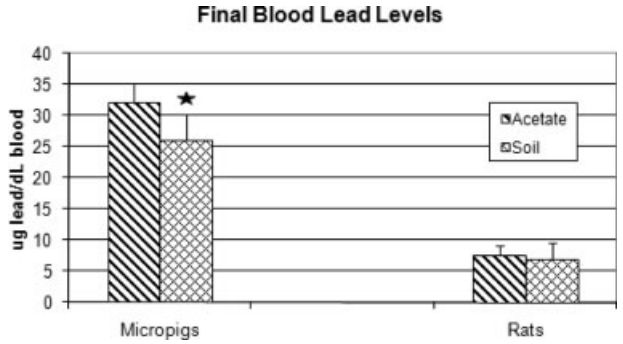

Fig. 2. Final blood lead levels. Values represent the mean ± SD for each group of five animals. An * indicates that the values are significantly different between groups at $P < 0.05$ using Dunnett's test for statistical significance.

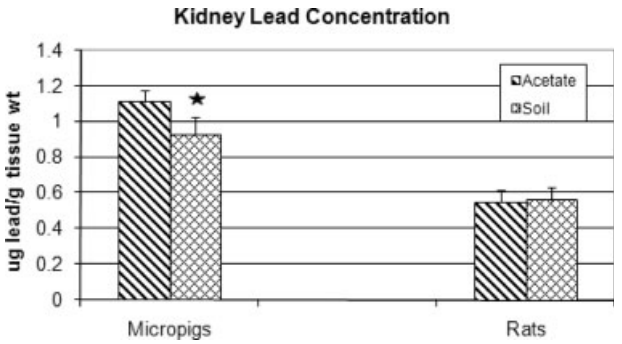

Fig. 4. Kidney lead concentrations. Values represent the mean ± SD for each group of five animals. An * indicates that the values are significantly different between groups at $P < 0.05$ using Dunnett's test for statistical significance.

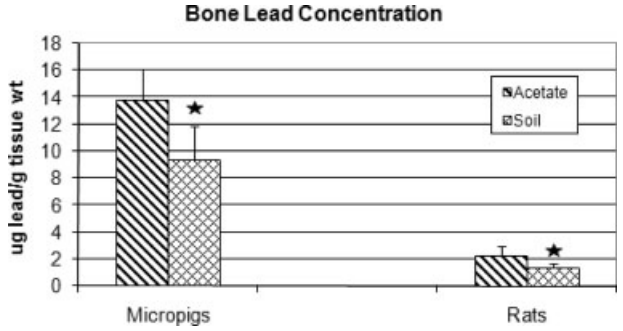

Fig. 5. Bone lead concentrations. Values represent the mean ± SD for each group of five animals. An * indicates that the values are significantly different between groups at $P < 0.05$ using Dunnett's test for statistical significance.

weight, weight gain, food consumption, food efficiency, and tissue lead concentration were analyzed using a SAS statistical software program for Dunnett's *T*-test for variability. Statistical significance for differences between groups was judged at $P < 0.05$.

Relative lead bioavailability was determined by comparing the tissue lead concentrations for the test soil study group with the respective lead acetate group, which was considered to be the reference compound for 100% bioavailability at the time of this study.

RESULTS

Test soils were obtained from Hazen Research, Golden, CO (Project No. 7910-08). These samples were taken from Weyerhaeuser sites in Tacoma, Washington. Test soil names (and reported lead concentrations) used in the study were labeled "S" (135 μg Pb/g) and "P" (1000 μg Pb/g). Analytical characterizations on soil samples for this study are shown in Table I. All screened soil samples (−60 mesh) show minimal variations in soil type according to the following physical characteristics: soil pH, carbonate content, clay content, and organic matter content.

Rat Studies

The results demonstrate a normal growth in body weights for all study groups and that there were no significant group-specific effects on rat body weights. Results showed an initial rapid rate of body growth (mean daily body weight gain) for all study animals, which plateaued off as the animals got older. As the animals got older, they increased their food consumption.

The weekly diet lead intake was calculated by multiplying the total amount of diet (g) consumed that week by the diet lead concentration (μg Pb/g diet). In general, the weekly dietary lead intake increased as the rats grew and ate more food. Results also showed that there was less dietary lead intake in the test soil group than the lead acetate group, although the difference was not statistically significant.

The final weekly-pooled, cleaned, dry fecal samples were then analyzed for lead concentration. Since the analytical fecal lead concentrations were determined on the dry fecal samples, the total fecal lead output was calculated by multiplying the fecal dry weight (g) by the fecal lead concentration (μg Pb/g dry weight). Results indicated that there

TABLE I. Analytical results on soil samples

Sample Label	Residual Moisture[a] (%)	Pb[b] (mg/kg)		Organic C[c] (%)		CO_2[d] (%)		Soil pH[e]	Clay[f] (%)	
		Assay[g]	Actual[h]	Assay[g]	Actual[h]	Assay[g]	Actual[h]			
S										
(A)	11.7	130	115	18.5	16.3	0.56	0.49	4.90	12.1	10.7
(B)	–	140	124	–	–	–	–	–	–	–
P										
(A)	14.6	1010	863	19.9	17.0	0.62	0.53	4.97	12.3	10.5
(B)	–	1000	854	–	–	–	–	–	–	–
(R)[i]	–	990	845	20.1	17.2	–	–	–	–	–

[a] Residual moisture is percent moisture in the −60 mesh material (after air-drying and screening). All assays were conducted on dried samples; therefore, the actual assays of the −60 mesh material must be adjusted accordingly (down).

[b] Pb analyses were conducted on two head samples (labeled A and B) by acid digestion and atomic absorption. All other assays were conducted on only one head sample (labeled A).

[c] Organic carbon determined by difference between total carbon (determined gravimetrically) and carbonate carbon.

[d] Carbonate carbon was determined by coulometric analysis.

[e] Soil pH was determined on 10% solids slurry in demineralized water.

[f] Clay content was determined by wet screening about a 5 g portion first through a 25-μm screen, then through a 10-μm screen using a dispersant (5 μm is preferred, but the material would not screen at 5 μm). The −10-μm portion is considered to be clay.

[g] Assay is dry basis.

[h] Actual is wet basis.

[i] (R) is a QA/QC repeat.

TABLE II. Whole body lead uptake

Animal	Soil Lead Diet	Total Diet Intake (g)	Total Lead Intake (mg Pb)	Fecal Lead Output (mg Pb)	Relative Whole Body Lead Uptake (%)
Rat	PbAc	504.8 ± 46.0	21.7 ± 2.0	18.2 ± 2.2	17.8 ± 4.7
Rat	Soil	497.0 ± 30.7	19.4 ± 1.2	16.5 ± 1.1	14.8 ± 3.2
Micropig	PbAc	21 926.2 ± 2906.9	911.2 ± 125.5	570.2 ± 76.9	37.3 ± 4.0
Micropig	Soil	22 891.5 ± 3557.3	948.4 ± 151.2	575.0 ± 192.2	40.8 ± 12.7

Values were calculated for the entire 30-day period. Total diet lead intake was calculated by taking the sum of the total weekly food consumed (g) times the soil-diet lead concentration (μg Pb/g). Total fecal lead output was calculated by taking the sum of the total weekly fecal dry weight (g) times the fecal lead concentration (μg Pb/g). Relative whole body lead uptake was calculated by taking the total whole body lead absorption (total diet lead intake minus total fecal lead output) divided by the total diet lead intake times 100. Values represent the mean ± SD for each group of five animals.

were no significant differences in fecal lead concentration between study groups.

One of the objectives for which these studies were designed was to test the intestinal absorption of lead from a test soil of unknown chemical matrix and compare that with the lead acetate reference compound. The results for relative whole body lead uptake were calculated by dividing the whole body lead absorption (retention) by the total diet lead uptake times 100. Whole body lead absorption was first calculated by subtracting the total fecal lead output from the total diet lead intake. In general, results demonstrated that for rats, there was a time-dependent decrease in relative whole body lead uptake from ~40% at week 1 to ~20% at week 4 for both test soil and lead acetate. Table II shows that there was no significant difference between study groups.

Micropig Studies

The data demonstrated a normal growth in micropig body weights for both study groups, although there were no significant study group-specific effects on body weights. The data for mean daily body weight gain were calculated by subtracting the week's beginning body weight value from the week's ending body weight divided by the applicable number of weekdays. Results demonstrate that there were no significant study-specific effects on mean body weight gain.

There was an increased mean daily food consumption for all study animals, although there were no significant group-related effects on mean daily food consumption. The weekly lead intake was calculated by multiplying the appropriate diet lead concentration (μg Pb/g diet) by the total amount (g) of diet consumed that week. In general, the results demonstrated that the weekly dietary lead intake increased as the micropigs grew and ate more food, although there were no significant differences between study groups.

Representative aliquots of feces were collected daily and sample wet weights were recorded. It was found that a 24-h drying period was sufficient to give a stable fecal dry weight of the aliquots. The dried fecal samples were then pooled into weekly samples and were sufficiently clean to be analyzed for lead concentration directly.

Since the analytical fecal lead concentrations were determined on the dry fecal samples, the total fecal lead output in mg Pb for rats was determined by multiplying weekly fecal dry weight by weekly fecal lead concentration (μg Pb/g dry weight). Similarly, the total fecal lead output for the micropigs in mg Pb was calculated by multiplying the fecal lead concentration by the total fecal weight "wet," divided by the micropig fecal wet/dry weight ratio. Results showed that there were no significant changes in total fecal lead output for both rats and micropigs, either as a function of time or between study groups.

Results for relative whole body lead uptake demonstrated that there were no significant differences between lead acetate or test soil study groups, nor were there any significant differences between the two animal species. The large 20-fold increases in total lead intake and the large 25-fold increase in total fecal lead output in micropigs as compared to rats have been reduced to minor differences, less than 30%, in relative whole body lead uptake between micropigs and rats.

Analytical Results

Animals, both rats and micropigs, were randomly selected from their respective lead acetate (1000 μg Pb/g soil-lead) study groups to determine blood lead equilibrium. Blood from both sets of animals was drawn on days 3, 7, 14, 21, and 28. Although control levels were not measured in the micropig, rats eating the control basal lead diet of 6.75 μg Pb/g diet (Soil "S," 135 μg Pb/g soil) were previously shown to have initial blood lead concentrations of 1.8 ± 1.3 μg Pb/dL (Smith et al., 2008). By day 3, both sets of animals on the 1000 μg Pb (acetate)/g soil-lead had reached comparable levels of 22–26 μg Pb/dL blood (Fig. 1). Thereafter, rat blood lead levels dropped rapidly at about week 1 and appeared to stabilize by about week 2 at ~9 μg Pb/dL. However, micropig blood lead levels increased gradually over time and tended to level off by about week 4 at ~36 μg Pb/dL. By the fourth week, the micropig blood lead levels were approximately four-fold higher than those in the

rats, although both species of animals received the same dosage of dietary lead.

Final blood lead concentrations, obtained at the time of sacrifice, confirmed a four- to six-fold increase in micropig blood lead concentrations as compared to rats. Results also demonstrate that there were no significant differences between lead acetate and test soil study groups in the rats. However, the final blood lead concentrations in the micropigs were significantly lower in the test soil study group as compared to the lead acetate group (Fig. 2).

In general, the results shown in Figure 3 demonstrate that there were no significant differences in liver lead concentrations between study groups for rats nor micropigs. Results also demonstrated that there were no significant differences in the kidney tissue lead concentrations for the rats from either study group. However, in micropigs, the kidney lead concentrations from the test soil group were significantly lower than those in the lead acetate group (Fig. 4). Kidney lead concentrations from the micropigs were two-fold higher than those in the rats.

Furthermore, there were significantly decreased bone lead concentrations (μg Pb/g tissue dry weight) for both rats and micropigs from the test soil study groups, as compared to their appropriate lead acetate study groups (Fig. 5). The increased sensitivity of the micropigs compared to the rats fed the same concentrations of lead, was further established by the six to seven times higher lead concentrations in micropig bones than in rat bone. This was consistent with the higher blood lead levels in the micropigs as compared to the rats, as described earlier.

In determining tissue lead relative bioavailability, the tissue lead concentrations in the animals from the test soil study group were compared to the tissue lead concentrations in the animals from the lead acetate study group. Lead acetate was considered to be the 100% reference for lead bioavailability at the time of study design. However, the 100% reference was taken as the mean tissue lead concentration from the lead acetate group ($n = 5$ animals per group). Appropriately, the comparisons to the test soil group were calculated as the group mean tissue concentration and not on an individual animal basis. The blood lead relative bioavailabilities of the test soil compared to lead acetate between animal species were very comparable, with 81 and 88% for the micropigs and rats, respectively. Similarly, the bone lead concentrations indicated a decreased lead bioavailability in the test soil compared to the acetate group. Again, the decrease was comparable between the two groups, with 68 and 62% for the micropigs and rats, respectively.

In contrast, only the micropigs demonstrated a significantly decreased tissue lead bioavailability for the test soil as compared to lead acetate in both the kidney and liver (83 and 87%, respectively), whereas, in the rats, the tissue lead concentrations between the two groups remained essentially unchanged ($\sim$100%).

DISCUSSION

Lead bioavailability, in general terms, can be defined as that portion of ingested lead that is retained by the body to interact with the various ongoing metabolic processes. A number of chemical, physical, and physiological factors, including Ca^{2+}, Fe^{2+}, diet composition, gut pH, *etc*., are involved in the absorption of ingested lead into the body and deposition in various tissues (Barltrop and Khoo, 1975; Barltrop and Meek, 1975; Flanagan et al., 1982; Heard et al., 1983: Hemphill et al., 1991; Mielke and Heneghan, 1991). Since nutritional and physiological processes are easier and cheaper, to study in rats than in larger mammals, many health regulatory guidelines for humans have been established based on studies done in rats. However, the interpretation of lead bioavailability studies in rats and lagomorphs needs to be viewed with caution for a number of reasons, including coprophagic and continuous feeding behavior in these species, gastrointestinal anatomy and acid secretion, high biliary excretion of lead in rats, and difficulty in assessing important developmental absorption mechanisms (Weis and LaVelle, 1991).

One objective is to find the appropriate, physiologically-relevant animal model to extrapolate to the human response. Nonhuman primates may provide even a closer model to the human response to lead bioavailability; indeed, monkeys have also been used in lead intoxication studies (Allen et al., 1974). However, many of these studies have focused on a more neurological and behavioral perspective, primarily because they are so close to humans in that respect.

Scientific evidence seems to suggest that, for several physiological processes (*i.e*., hematological, nutritional, and cardiovascular response), pigs may be more physiologically relevant to the human condition than do rats (Dodds, 1982; Miller and Ullrey, 1987; LaVelle et al., 1991). In addition to this, Casteel et al. (1997) demonstrated the use of young pigs in a short-term feeding study to measure the lead bioavailability from lead-contaminated soils. They examined the molecular species and the particle size distribution of the lead, which was present in the contaminated soils. They also demonstrated that the nonlinear increase in blood Pb concentrations closely mimic the blood lead accumulation seen in young children.

The goals of this particular task presented here were to (1) further develop the lead feeding model in juvenile Yucatan micropigs; (2) compare the lead bioavailability from lead-contaminated soils between these micropigs and a previously characterized rat feeding model (Smith et al., 2008); and (3) compare the lead uptake from a test soil of known lead concentration with a lead acetate standard, which was presumed to be 100% bioavailable at the time of the study.

In this task, both Harlan Sprague–Dawley rats and Yucatan micropigs were fed lead-contaminated soil as a 5% (w/w) mixture with their diet. The lead-contaminated soil was

either a specific test soil of known lead concentration (1000 μg Pb/g) or a basal, low lead soil (135 μg Pb/g), which was spiked with lead acetate to match the lead content of the test soil. Lead acetate was used as the reference compound, which was considered to be 100% bioavailable at the time of study. Since the lead-contaminated soil was mixed 5% (w/w) with their diet mixture, which was specially formulated to fulfill 100% of their nutritional requirements at a 95% dilution, the effective diet lead concentration was 50 μg Pb/g diet. This level of lead was chosen because in a previous phase of this study (Smith et al., 2008), soil-lead concentrations up to 3000 μg Pb/g were used without any apparent lead toxicity, and the final blood lead levels were linear within this concentration range. In agreement with the results from the previous phase of this study, this level of dietary lead did not exhibit any adverse effects in rats, as determined by body weight gain, food consumption, or fecal output. In the initial pretest phase of this study, several of the micropigs did exhibit diarrhea as a result of getting used to the semipurified diet mix. However, as further evidence for the absence of any apparent toxicological effects, the micropigs did not exhibit any significant study-specific effects on the fecal wet/dry weight ratio throughout the test period.

To compare lead uptake between rats and micropigs, tissue lead concentrations were analyzed in blood and several target organs known to accumulate lead. In general, when lead is absorbed, it is thought to exist in at least three separate metabolic, yet highly interactive pools, namely blood, bone, and soft tissues (particularly the liver and kidneys). Our results showed that initially (day 3), rats demonstrated a high blood lead level ($\sim$25 μg Pb/dL), which then rapidly decreased to 11 μg Pb/dL at the end of week 1, and appeared to stabilize after week 2 to about 9 μg Pb/dL. This may have been related to a decreased absorptive capacity as the animals reached a steady-state level with interaction between the different metabolic pools. Conversely, in the micropigs, the blood lead levels gradually increased over time but tended to stabilize by week 4. Final blood lead levels at day 30 confirmed that micropig blood lead concentrations were four- to six-fold greater than those in the rats, which had consumed the same lead concentration (50 μg Pb/g diet). Results demonstrated that the blood lead levels in the micropigs from the test soil study group were significantly lower than those in the lead acetate study group. In contrast, rats did not exhibit any significant differences between study groups.

Liver tissue lead concentrations were seven-fold greater in the micropigs as compared to those in rats (Fig. 3). Although results indicated that micropigs from the test soil study group had a decreased liver lead concentration than what the lead acetate study group had, this difference was not statistically significant. In addition, rats did not demonstrate any significant difference between study groups.

Results also demonstrated two-fold higher kidney lead concentrations in the micropigs as compared to the rats (Fig. 4). Again, only in the micropigs did the test soil cause a significantly decreased lead incorporation than the lead acetate study group. Rats did not demonstrate any significant difference between study groups.

Comparisons of the bone lead concentrations between rats and micropigs indicated at least a six- to seven-fold greater incorporation of lead into the micropigs as compared to the rats (Fig. 5). Also, results demonstrated that micropigs from the test soil study group had a significantly decreased bone lead concentration than what the lead acetate study group had. In contrast, rats did not demonstrate any significant differences between study groups.

In summary, these studies demonstrate the superiority of micropigs over rats when it comes to lead bioavailability feeding studies for a number of reasons. Although we did try to eliminate coprophagic activity in the rats by using raised wire mesh flooring, there were other factors that were not easily corrected, such as the high biliary excretion of lead in rats. Micropigs did demonstrate greater tissue lead concentrations (μg Pb/g tissue weight) than rats receiving similar lead concentration diets. These higher tissue lead concentrations in the micropigs allowed the differences between treatment groups to be more easily demonstrated. The blood and bone lead concentrations from both species of animals demonstrated that the test soil was less bioavailable than the lead acetate reference compound; however, it depended on the specific tissue being examined as to the extent of the decreased bioavailability: 81–88% and 62–68% for the blood and bone, respectively. In addition, the micropigs were the only test animals that demonstrated a decreased bone, blood, liver, and kidney relative bioavailability of the test soil compared to the lead acetate reference compound.

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